

WHAT IS CLAIMED IS:

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2 a1 7
3 1. An *in vitro* system capable of recapitulating regulated RNA turnover of an exogenously added preselected target RNA sequence comprising a cell extract and said target RNA sequence.

B 1 11
B 2 2. The system of claim 1 wherein said regulated RNA turnover is selected from the group
AB3 consisting of AU-rich element regulated RNA turnover and C-rich element regulated RNA turnover. ^{deadenylation and degradation}
RNA deadenylation and degradation ^{deadenylation and degradation}

1 3. The system of claim 1 wherein said cell extract is isolated from lysed eukaryotic cells or
2 tissues.

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c3 4. The system of claim 1 wherein said cell extract is obtained from a cell line selected from the group consisting of HeLa cells and a T cell line.

1 5. The system of claim 1 wherein said cell extract is prepared from cells comprising
2 foreign nucleic acid.

a1 1 6. The system of claim 1 wherein said cell extract is prepared from cells which are ^{uninfected,}
2 stably transfected, or transiently transfected.

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a2 7. The system of claim 1 wherein said cell extract is partially purified.

8. The system of claim 1 wherein said cell extract is depleted of activity of proteins that bind polyadenylate. B

B 1 9. The system of claim 1 wherein said cell extract depleted of activity of proteins that bind
2 polyadenylate is prepared by a method selected from the group consisting of:

3 (a) addition to said system of polyadenylate competitor RNA;

4 (b) sequestration of proteins that bind polyadenylate;

a5 (c) addition of a proteinase that inactivates a protein that ^{binds} bind to polyadenylate; and

6 (d) addition of an agent that prevents the interaction between polyadenylate and an
a 7 endogenous macromolecule that binds to polyadenylate.

1 10. The system of claim 9 wherein said sequestration of proteins that bind polyadenylate
a 2 is achieved by treatment of said extract with ^aan material that depletes
3 macromolecules that bind polyadenylate selected from the group consisting of
4 antibodies to proteins that bind polyadenylate, polyadenylate, and the combination
5 thereof.

1 11. The system of claim ⁹10 wherein said material is attached to a matrix.

a 1 12. The system of claim 1 wherein said target RNA sequence is selected from the group ^{consisting}
2 of synthetic RNA, naturally occurring RNA, messenger RNA, chemically modified
3 RNA, and RNA-DNA derivatives.

1 13. The system of claim 12 wherein said target RNA sequence comprises a 5' cap and a
2 3' polyadenylate sequence.

1 14. The system of claim 1 wherein said target RNA sequence is selected from the group
2 consisting of unlabeled target RNA sequence, labeled target RNA sequence, and the
3 combination thereof.

1 15. The system of claim 14 wherein said labeled target RNA sequence is labeled with a
2 moiety ~~is~~ selected from the group consisting of a fluorescent moiety, a visible
3 moiety, a radioactive moiety, a ligand, and a combination of fluorescent and
4 quenching moieties.

1 16. The system of claim 1 additionally comprising exogenously added nucleotide
2 triphosphate.

1 17. The system of claim 16 wherein said nucleotide triphosphate is ATP.

1 18. The system of claim 1 further comprising a reaction enhancer.

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The system of claim 18 wherein said reaction enhancer is selected from the group consisting of polyvinyl alcohol, polyvinylpyrrolidone and dextran.

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The system of claim 19 wherein said reaction enhancer is polyvinyl alcohol.

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21.

A method for identifying an agent capable of modulating the stability of a target RNA sequence comprising

in-vivo

- (A) providing the system of claim 1;
(B) introducing said agent into said system;
(C) determining the extent of ~~turnover~~ *deadenylation and degradation* of said target RNA sequence; and
(D) identifying an agent able to modulate the extent of said turnover as capable of modulating the stability of said target RNA sequence.

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The method of claim 21 wherein said system additionally comprises nucleotide triphosphate.

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The method of claim 22 wherein said nucleotide triphosphate is ATP.

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The method of claim 21 wherein said agent is an RNA stability modifying molecule.

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The method of claim 21 wherein said target RNA sequence is selected from the group consisting of unlabeled target RNA sequence, labeled target RNA sequence, and the combination thereof.

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The method of claim 25 wherein said labeled RNA sequence is labeled with a moiety ~~is~~ selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

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The method of claim 21 wherein said ~~monitoring~~ *determining* the extent of ~~turnover~~ *deadenylation and degradation* of said target RNA sequence comprises determining the extent of degradation of said labeled target RNA *sequence*.

1 28. The method of claim 21 wherein said modulating the stability of a target RNA
2 sequence increases the stability of said target RNA sequence.

1 29. The method of claim 21 wherein said modulating the stability of a target RNA
2 sequence decreases the stability of said RNA sequence.

1 ²³ 30. The method of claim ¹⁵ 21 wherein said agent is capable of modulating the activity of a
2 AU rich element binding protein or a C-rich element binding protein.

1 31. The method of claim 30 wherein said AU rich element binding protein is selected
2 from the group consisting of a member of the ELAV protein family; AUF1;
3 ^{sup} _{c10} tristetrapolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP
4 A1; AU-A; and AU-B.

1 ²⁵ 32. The method of claim ²⁴ 31 wherein said member of the ELAV protein family is
2 selected from the group consisting of HuR, Hel-N1, HuC and HuD.

1 33. A method for identifying an agent capable of modulating the ^{in-vivo} stability of a target
2 RNA sequence in the presence of an exogenously added RNA stability modifier
3 ^{sup} _{c11} comprising

4 (a) providing the system of claim 1;

5 (b) introducing said RNA stability modifier into said system;

6 (c) introducing said agent into said system;

7 (d) determining the extent of ^{deadenylation and degradation} turnover of said target RNA sequence; and

8 (e) identifying an agent able to modulate the extent of said ^{deadenylation and degradation} turnover as capable
9 of modulating the stability of said target RNA sequence in the presence of
10 said exogenously added RNA stability modifier.

1 34. The method of claim 33 wherein said system additionally comprises nucleotide
2 triphosphate.

1 ^{sup} _{c12} 35. The method of claim 34 wherein said nucleotide triphosphate is ATP.

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36. The method of claim 33 wherein said target RNA sequence is selected from the group consisting of unlabeled target RNA sequence, labeled target RNA sequence, and the combination thereof.

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37. The method of claim 36 wherein said labeled RNA sequence is labeled with a moiety is selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

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38. The method of claim 33 wherein said determining the extent of turnover of said target RNA sequence comprises determining the extent of degradation of said labeled target RNA.

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39. The method of claim 33 wherein said RNA stability modifier increases the stability of said target RNA sequence.

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40. The method of claim 39 wherein said agent decreases the stability of said target RNA sequence increased by said RNA stability modifier.

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41. The method of claim 33 wherein said RNA stability modifier decreases the stability of said target RNA sequence.

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42. The method of claim 41 wherein said agent increases the stability of said target RNA sequence decreased by said RNA stability modifier.

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43. The method of claim 38 wherein said agent is capable of modulating the activity of a AU rich element binding protein or a C-rich element binding protein.

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44. ~~The method of claim 43 wherein said AU rich element binding protein is selected from the group consisting of a member of the ELAV protein family; AUF1; tristetrapolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP A1; AU-A; and AU-B.~~

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The method of claim 44 wherein said member of the ELAV protein family is selected from the group consisting of HuR, Hel-N1, HuC and HuD.

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46.

A method for identifying an agent capable of modulating the deadenylation of a target RNA sequence comprising

- (A) providing the system of claim 1 in the absence of a nucleotide triphosphate;
- (B) introducing said agent into said system;
- (C) monitoring the deadenylation of said target RNA sequence in said system; and
- (D) identifying an agent able to modulate the extent of said deadenylation as capable of modulating the deadenylation of said target RNA sequence.

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A method for identifying an agent capable of modulating ^{regulated} the deadenylation and degradation of a target RNA sequence comprising

- (A) providing the system of claim 1 in the presence of a nucleotide triphosphate;
- (B) introducing said agent into said system;
- (C) monitoring the deadenylation and degradation of said target RNA sequence in said system; and
- (D) identifying an agent able to modulate the extent of said deadenylation and degradation as capable of modulating the deadenylation and degradation of said target RNA sequence.

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A method for identifying an agent capable of modulating cell growth or cell differentiation in a mammal comprising determining the ability of said agent to modulate the stability of a target RNA sequence involved in the modulation of cell growth or differentiation in accordance with claim 21.

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The method of claim 48 wherein said agent capable of modulating cell growth or cell differentiation intervenes in cellular transformation.

1 50. The method of claim 48 wherein said agent ~~capable of modulating cell growth or cell~~
2 differentiation intervenes in immune dysregulation.

1 51. A method for identifying, characterizing or isolating an endogenous molecule
2 suspected of participating in the deadenylation or degradation of RNA or regulation
3 thereof comprising
4 (A) providing the system of claim 1;
5 (B) introducing said ~~protein~~ ^{endogenous molecule} suspected of participating in the regulation of
6 RNA ~~turnover~~ ^{deadenylation and degradation} into said system;
7 (C) monitoring the stability of said target RNA sequence in said system; and
8 (D) identifying, characterizing or isolating said endogenous molecule able to
9 modulate said deadenylation or degradation as capable of participating in
10 the deadenylation or degradation of RNA or regulation thereof.

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2 52. The method of claim ~~51~~ ³⁹ wherein said molecule suspected of participating in the
3 deadenylation or degradation of RNA or regulation thereof is protein or RNA.

1 53. A kit for monitoring the stability of a preselected target RNA sequence under
2 conditions capable of recapitulating regulated RNA turnover, said kit comprising:
3 (a) cell extract ^{supernatant} depleted of activity of proteins that bind polyadenylate;
4 (b) other reagents; and
5 (c) directions for use of said kit.

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2 54. The kit of claim ~~53~~ ⁴⁴ further comprising nucleotide triphosphates, a reaction enhancer,
3 a target RNA sequence, or any combination thereof.

1 55. A method for identifying an agent capable of modulating the ^{of} degradation ^{of} a target
2 RNA sequence in the absence of deadenylation comprising
3 (A) providing a cell extract in the presence of a nucleotide triphosphate;
4 (B) introducing said agent into said cell extract; and
5 (C) monitoring the degradation of said target RNA sequence in said extract.